Analysis of MCL-1 Copy Number Variation in Non-Small Cell Lung Cancer

Almahdi Alzaroukh Jaber[1], Howard Pringle[2], David Guttery[3], Aesa Alzaroug Jaber[4]

[1]Lecturer, Department of Pathology and Clinical Pathology, Faculty of Veterinary Medicine, Tripoli University, Libya, [2]Expert Academic Pathologist, Department of Cancer Studies And Molecular Medicine, University of Leicester, [3]Lecturer, Department of Cancer Studies And Molecular Medicine, University of Leicester, [4]Assistant Professor, Department of Surgical Science, University of Science and Technology of Fujairah, UAE

Abstract

Amplification of the MCL-1 gene induces MCL-1 overexpression, which is an important marker for drug resistance in several malignancies through evasion of apoptosis. We analysed 39 lung adenocarcinomas (ADCs), 38 lung squamous cell carcinomas (SCCs), two large cell lung carcinomas (LCCs) and cell lines. A duplex ratio test TaqMan qPCR approach was performed for MCL-1 copy number change analysis and we demonstrated amplification of MCL-1 in H1395 cell line and copy number gain (isochromosome) in H2342 cell line. Immunohistochemistry (IHC) and *in situ* hybridization (ISH) were performed for protein and mRNA analysis respectively. MCL-1 amplification was detected in 13% and 2.5% of SCCs and ADCs respectively. *MCL-1*-amplified tumours demonstrated high expression of MCL-1 mRNA. Copy number gain was detected in 2.5 % of ADC but not SCC.

Key Words: *Non-small cell lung cancer, Amplification, Gain, MCL-1*

Introduction

Lung cancer is the leading cause of cancer death worldwide[1] (Imielinski *et al*., 2012) accounting for approximately 28% of all cancer-related deaths[2] (Huncharek *et al*., 1999). Non-small cell lung cancer (NSCLC) is the most common type of primary lung cancer, accounting for approximately 85% of new diagnoses every year and can be classified into two major histologic subtypes which are adenocarcinoma (ADC) and squamous cell carcinoma (SCC)[3] (Dearden *etal*., 2013). The *MCL-1* gene encodes for the MCL-1 protein[4] (Wei *et al*., 2012), which is an anti-apoptotic protein, belonging to the BCL-2 family and represents one of the most commonly amplified genes in human cancers[5] (Perciavalle *et al*., 2012) with a high prevalence in lung cancer[6] (Beroukhim *et al*., 2010). Analysis of 300 individual tumours using next-generation sequencing has shown that *MCL-1* is one of the most commonly amplified genes in human malignancies[7] (Vogler, 2014), with several genetic alterations such as translocations and copy number changes being identified in lung cancer[8]., (Staaf *et al*., 2013).

Quantitative real-time PCR (qRTPCR) is an extremely sensitive method for detection of somatic mutations (even at single copy frequencies) and copy number alterations, making this and attractive method for screening clinical biopsies and minute lysates from micro-dissected samples. Furthermore, high throughput and low cost assays are other advantages of qRTPCR, as well as the ability to multiplex[9-10] (Nicklas and Buel, 2003, Xu *et al*., 2012). Taken together, this offers a huge advantage over WGS, which is plagued by high costs of consumables, laborious workflows and relatively lowers sensitivity. Overexpression of MCL-1 correlated with chemo-resistance, and reduced apoptosis in NSCLC cells was identified[11] (Song et al., 2005), however there is no significant correlation between MCL-1 and patient outcome[12] (Wesarg et al., 2007). MCL-1 protein is known to have a short half-life[4] (Wei et al., 2012). MCL-1 expression was positively correlated with gene amplification[13] (Balko et al., 2014).

Materials and Methods

Primer design for copy number analysis

Prior to primer and probe design, the genes of interest were checked on the Sanger Institute website (https://cancer.sanger.ac.uk/cell_lines/conan/search). Using the cell line copy number project database (Conan), appropriate cell lines with copy number changes were selected. These amplifications have been validated by the Sanger Institute. After the amplification site was determined, the Primer 3.0 software (v. 4.0.0) was used to design PCR primers and probes. All probes and primers were blasted on the NCBI databases to ensure their specificity to the target gene. Primers and probe temperatures were optimized using Primer 3 express (Applied Biosystems). The assays were obtained from the manufacturing company as lyophilized powder, and according to the manufacturer recommendations the powder was dissolved in sterile ultrapure water under sterile conditions to obtain stock primers and probes at concentrations of 100 µM. 10 µM aliquots of primers and 2 µM aliquots of probes were then prepared and stored at -20°C. All used probes were FAM-MGB or VIC-labelled. *MCL-1*forward primer, *MCL-1*reverse primer and two VIC and FAM-labelled MGB probes were designed. Two different reference genes were generated; one on the p-arm (*H6PD*) and the other on the q-arm of the chromosome (*CCT3*) with MCL-1. MCL-1 copy number analysis assays are listed in table1. A duplex ratio test was used to determine copy number changes. Three different master mixes were prepared, each containing a FAM-labelled probe for the target of interest, with the other VIC-labelled for the reference. The reaction was run as follows*: MCL-1* vs *H6PD1* (Ref 1), *MCL-1* vs *CCT3* (Ref 2) and Ref 1 vs Ref 2. *MCL-1* amplification was analysed and discriminated from gain as follows:

- *MCL-1* CT value – *H6PD* CT value
- *MCL-1* CT value – *CCT3 CT* value
- *CCT3* CT value – *H6PD* CT value

Table1. This table show list of primers used for analysis of MCL-1 copy number variation.

Primer	Sequence 5'→3'	Length (nt)	Tm (°C)	Supplier
MCL-1 F	GCATCGAACCATTAGCAGAAAGT	23	58.5	Sigma
MCL-1 R	GCCAGTCCCGTTTTGTCCTT	20	59.9	Sigma
MCL-1 probe	VIC-TCACAGACGTTCTCG-MGB	15	68.0	AB
MCL-1 probe	FAM-TCACAGACGTTCTCG-MGB	15	68.0	AB
CCT3 F	FAM-GCATCATTGAAGACTCCTGTGTCT-MGB	24	58.4	Sigma
CCT3 R	GCATACGTGGATGGGTCACAT	21	59.1	Sigma
CCT3 probe	FAM-CGTGGAGTCATGATTA-MGB	16	67.0	AB
H6PD F	TTCAGGCCAGGAGAGAAGTCTT	22	58.2	Sigma
H6PD R	TTGTGCTGGTTGATATGAATGTGT	21	58.1	Sigma
H6PD probe	VIC-TTGTGCTGGTTGATATGAATGTGT-MGB	19	68.0	AB

Positive control DNAs for MCL-1 amplification and gain analysis were obtained from cell line H1395 and H2342 respectively. The experiment was run under standard thermo-cycling conditions (40 cycles, 60°C annealing temperature and 95°C denaturing temperature), after which the ΔCT between each gene pair was calculated. The tumour sample was then normalized to the control sample (Human genomic DNA) using ΔΔCT, and a Z-score was analysed using CT values of the normal adjacent tissue against the ΔΔCT.

Tumour samples

A total of 85 NSCLC tissues (LT1-LT85) were obtained from the University Hospital of Leicester (UHL) with consent and clinical outcome. Details of the tissues are listed in Table 2.

Table2. This table summarize the clinicopathological feature of patients used in this study

Clinicopathological feature		Total
Tumour type	Adenocarcinoma	39
	Squamous cell carcinoma	38
	Large cell carcinoma	2
	Atypical carcinoid tumour	2
	Adeno-squamous	1
	Combined ADC and SCLC	1
	Combined SCC and SCLC	1
	Combined large cells and small cells	1
Patient gender	Male	45
	Female	42
Patient age	0-44	2
	45-54	3
	55-64	14
	65+	67
Smoking history	Smokers	16
	Ex-smokers	26
	Non-smokers	3
	Unknown	41
Tumour stage	IA	8
	IB	26
	IIA	3
	IIB	21
	IIIA	18
	IIIB	1
	IV	3
	Unknown	7

DNA extraction

DNA was extracted from NSCLC samples using the QIAamp DNA blood mini kit from Qiagen (Cat No 51106). The fixed tissue was incubated at 65°C for 5 minutes, followed by immersion in xylene and graded alcohol. The tissue was dried and then scraped into a 1.5 ml eppendorf using an ATL buffer. Ten mg/ml proteinase K was added to the eppendorf and incubated at 56°C for 4 days. AL buffer was then added and the solution transferred to a Safe Lock tube and incubated for 10 minutes at 70ºC. Absolute ethanol was then added at room temperature. The mixture was transferred into a QIAamp column and centrifuged for 60 seconds at 8000 rpm, then the collection tube was changed and AW1 added to the column. The column was centrifuged again at the same speed for 1 minute, after which the collection tube was replaced and AW2 was added to the column. The column was centrifuged for 3 minutes at 14000 rpm. After discarding the buffer, the column was centrifuged for 60 seconds at the same speed. To elute the DNA, AE buffer was added to the column and incubated at room temperature for 5 minutes. The column was centrifuged at 8000 rpm for 1 minute and then the eluted DNA was transferred into a new Eppendorf and stored at 4ºC until required.

DNA quantification

DNA concentrations were measured using qPCR and a standard curve. An ALU repeat assay (table 3) was used to determine DNA concentrations in all samples. The first standard was 10 ng DNA with six subsequent standards produced using a dilution factor of 1:2. Reactions were performed in a final volume of 10 µL comprising 5 µL TaqMan Genotyping Master Mix, 0.6 µL F primer,0.6 µL R primers, 0.2 µL probe and 0.6 µL H_2O. The primer concentration was 10 µM and the probe concentration was 2 µM. 10 ng of DNA in a volume of 3 µL was added to make up the total volume of the reaction to 10 µL. Reagents and DNA were loaded into 96 well plates; the targeted region of the gene was amplified on a Step One Plus thermocycler (Applied Biosystems) using standard thermal cycling conditions. Reactions were run as duplicates or in triplicate according to the amount of DNA obtained from fixed tissues.

Table3. This table shows sequences of ALU repeat assays used for DNA quantification.

Primer	Sequence 5'→3'	Length (nt)	Tm (°C)	Supplier
ALU F	GACCATCCCGGCTAAAACG	19	59.0	Sigma
ALU R	CCACTACGCCCGGCTAATTT	20	59.9	
ALU_ probe	VIC-CCCGTCTCTACTAAA-MGB	15	65.0	

Immunostaining

Paraffin-embedded tissues from NSCLC patients and normal control samples from tumour-adjacent tissue were stained for MCL-1. Specimens were heated in an incubator for 10 minutes at 65˚C and then dewaxed and rehydrated by passing them through xylene and descending graded alcohol, respectively. The antigen retrieval method was citrate buffer/microwave antigen retrieval. A Novolink polymer detection system was used to develop the immuno-staining. Overnight incubation at 4˚C was performed for the tested antibody (AB). Rabbit polyclonal antibody (pAB) S-19(Cat No SC-819) was used in this study. Preliminary experiments for the antibodies were performed on tonsil tissues; various dilutions for the antibody starting from 1:100-1:2000 were tested to obtain appropriate concentrations, and TBS buffer containing 3% FBS/0.1% TritonX-100 was used as an antibody diluent to avoid AB non-specific binding. Bright field microscopy (Leica, MD, 2500, Applied Biosystems) was used to analyse the slides. Images were photographed using a Leica DFC 420 camera (Leica Microsystems Ltd., Switzerland). The tumour area was first marked and then H-score

was calculated using Aperio Image Scope software (v.11.1.2.760),with an overall score of 0-300 being stratified into 3 categories of % nuclear staining: weak positive, intermediate positive nuclear staining and strong positive staining nuclear staining (Cohen et al., 2012). Staining level was then categorised according to median of the all staining levels in tumour samples.

In situ hybridization technique (ISH) for MCL-1 mRNA expression analysis

ISH was used to determine the level of MCL-1-mRNA expression in NSCLC cases. Paraffin-embedded tissues from patients with NSCLC were analysed for MCL-1 mRNA expression along with different lung cancer cell lines with *MCL-1* amplification. Slides were heated to 60°C for an hour, and then passed twice through xylene solvent for 10 minutes, followed by merging twice into 100% fresh ethanol or 99% IMS for 3 minutes each time. Sections were dried for 5 minutes at room temperature. A hydrophobic barrier was created around the specimen using an ImmEdge pen (Vector Laboratories, Inc., Cat No. 4000; Burlingame, CA, USA) and then 5-8 drops of pre-treatment 1 were added onto the specimen and incubated at room temperature for 10 minutes. The pre-treatment 1 was decanted and the tissue sections immersed in distilled water with agitation. In a fume hood, pre-treatment 2 was heated to 100-104°C and slides were then transferred into boiling solution for 15 minutes. Samples were removed from the boiling pre-treatment 2 and rinsed in distilled water with agitation, followed by submerging into 100% or 99% IMS. Samples were then treated with pre-treatment 3 and incubated at 40°C for 30 minutes for the lung tissue and for 15 minutes for the cell lines. Samples were washed in distilled water with frequent agitation, which was repeated for 5-15 minutes. Samples were transferred into a warm HyEZ humidity control tray (40°C) containing a slide rack and humidifying paper. Target and control probes were pre-warmed at 40°C for 10 minutes and 3-4 drops of probe were added to the specimens, which were then incubated for 2 hours at 40°C in a HyEZ oven. 1X wash buffer was used immediately to wash the slides for 2 minutes after 2 hours incubation, and then the wash was repeated in fresh 1X wash buffer with frequent agitation. Following that, amplifiers 1, 2, 3 and 4 were applied to the slides, and the slides were incubated at 40°C for 30, 15, 30 and 15 minutes, respectively. Amplifiers 5 and 6 were then added for 30 and 15 minutes, respectively, and incubated at room temperature followed by washing twice for 2 minutes after each amplifier treatment. Equal amounts of DAB solution A and DAB solution B were mixed and then applied to the tissue sections for 10 minutes at room temperature to develop the signals. Distilled water was then used to wash the DAB chromogene and the slides submerged in 50% haematoxylin for 2 minutes. Fresh water was used to wash the haematoxylin stain, and then the slides were passed through 70% ethanol or 95% IMS once for 2 minutes and twice in 100% ethanol or 99% IMS for 2 minutes, followed by merging into fresh xylene for 5 minutes. Finally, slides were mounted using a DPX mounting medium (Cell Path Ltd., Newtown Powys, UK). Pictures were captured using a Leica DFC 420 camera (Leica Microsystems Ltd., Switzerland) and the staining was analysed using the Aperio Image Scope software (v.11.1.2.760).

Results

MCL-1 copy number variation analysis in tumour specimens Copy number variation analysis (amplification and gain) for *MCL-1*was performed using qPCR. Each tumour tissue was analysed using ΔΔCT and a Z score test was used to analyse the significance of the gain and amplification in the tumour samples at a confidence interval (CI) of 99.9%.

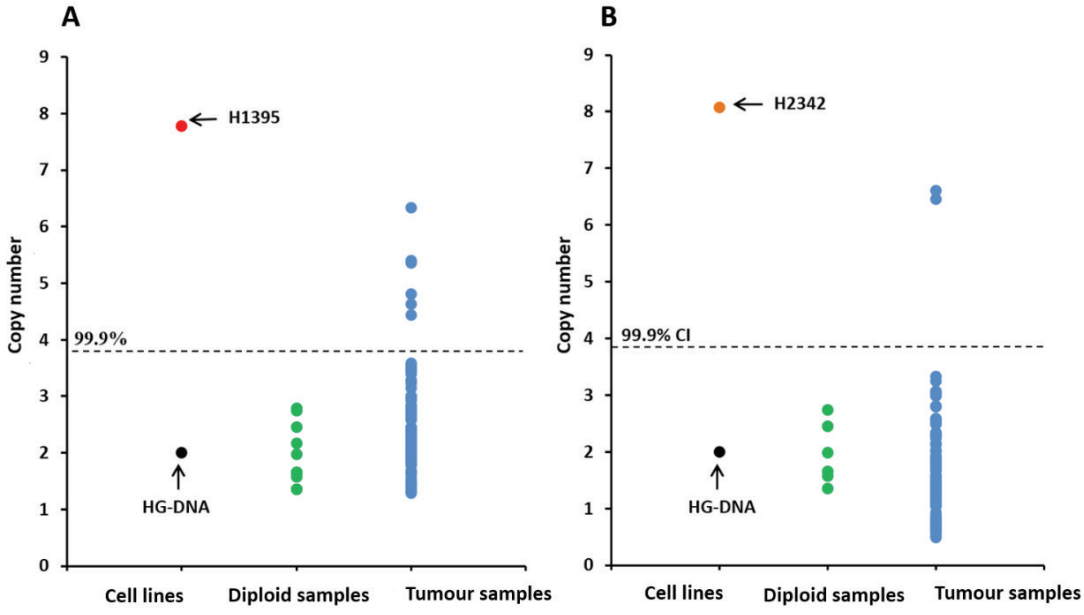

Figure 1. *MCL-1* amplification and gain in tumour samples.

MCL-1 amplification was significant in tumour samples LT5, LT7, LT8, LT9, LT17 and LT38 at a 99.9% confidence interval, diploid samples (tumour adjacent tissues) and HG-DNA did not show significant *MCL-1* amplification, whereas the positive control DNA from the H1395 cell line showed significant *MCL-1* amplification (p <0.001) and showed about eight copies of *MCL-1* (A). *MCL-1* gain was significant in tumour samples LT7 and LT26 at a 99.9% confidence interval; control tumour samples and HG-DNA did not show significant *MCL-1* gain, whereas positive control DNA from the H2342 cell line showed very significant *MCL-1* gain (p <0.001) (B).

MCL-1 amplification was found in 7.5% (6/79) of NSCLC cases. *MCL-1* amplification was found in 5/38 (13%) of SCC, however one patient with ADC demonstrated *MCL-1* amplification. Gain was detected only in two cases of NSCLC, one SCC which has focal amplification as well and one ADC (Table4). *MCL-1* amplification was detected in both early and late stages of NSCLC without correlation to stages of the disease. There was no significant difference between gains and amplifications according to the stage of the disease. In both males and females, and both smokers and non-smokers showed *MCL-1* amplifications. However, males had a higher frequency of *MCL-1* amplification (4/5) compared to females (1/5).

Table 4. Copy number variation in NSCLC. *MCL-1* copy number changes was detected in NSCLC. Six cases were amplified for *MCL-1*; the LT5 showed six copies of *MCL-1*, LT17 and LT38 showed five copies, whereas LT7, LT8 and LT9 showed the lowest copy number (four copies).

Sample ID	Histology	Gender	Stage	gene	Copy number change	No of copies
LT5	SCC	M	IB	MCL-1	Amplification	6
LT7	SCC	F	IB	MCL-1	Amplification/Gain	4/3
LT8	SCC	M	IIIA	MCL-1	Amplification	4

Cont... Table 4. Copy number variation in NSCLC. *MCL-1* copy number changes was detected in NSCLC. Six cases were amplified for *MCL-1*; the LT5 showed six copies of *MCL-1*, LT17 and LT38 showed five copies, whereas LT7, LT8 and LT9 showed the lowest copy number (four copies).

LT9	ADC	F	Unknown	MCL-1	Amplification	4
LT17	SCC	M	IIA	MCL-1	Amplification/Gain	5
LT26	ADC	F	IB	MCL-1	Gain	6
LT38	SCC	M	IB	MCL-1	Amplification	5

MCL-1 immunohistochemistry analysis

Immuno-staining analysis showed mild, and medium to high expression of MCL-1 in normal lung tissue and tumour samples (SCC, LCC and ADC) respectively. Normal tissue revealed mild and moderate expression of MCL-1 in air passages and macrophages, respectively and various levels of MCL-1 expression were identified in different histological subtypes of NSCLC (figure 2).

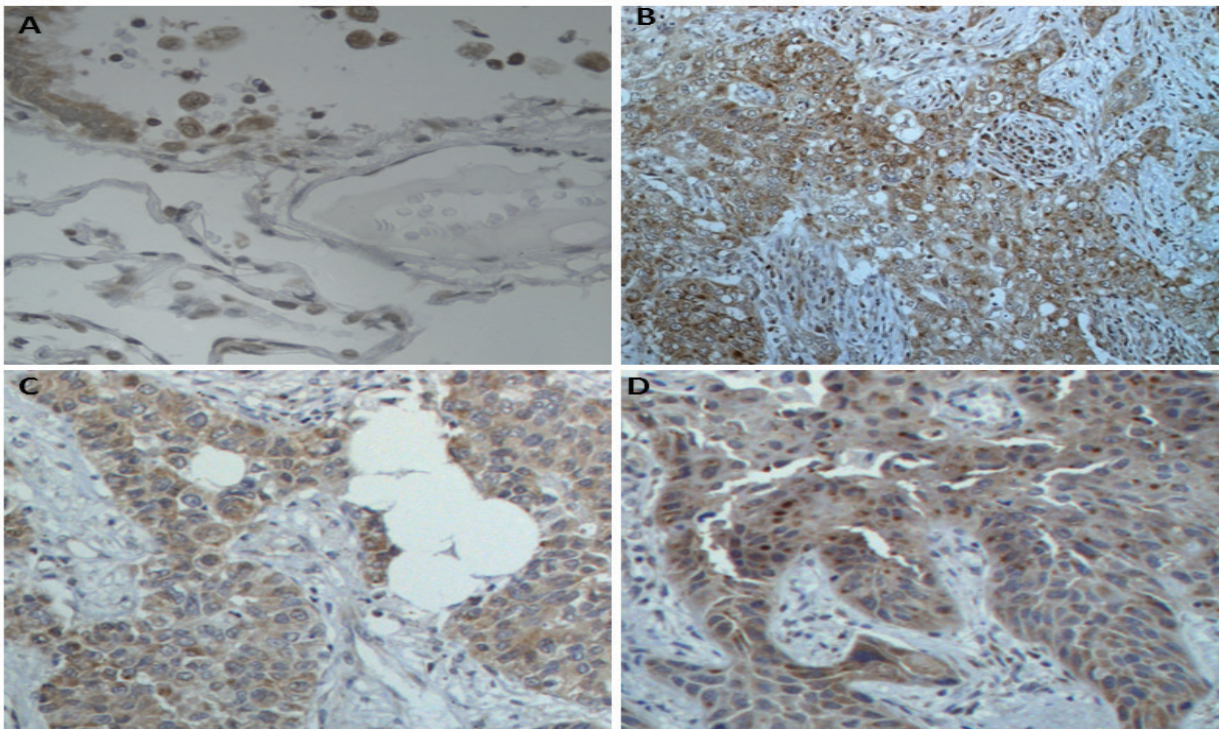

Figure 2. Expression of MCL-1 in normal and tumour lung tissue. MCL-1 was expressed by the epithelial lining of bronchioles and macrophages of normal lung (A). Strong positive staining of MCL-1 was detected in ADC (B) and SCC (D) samples with clear cytoplasmic location and a granular appearance of the MCL-1 protein in the mitochondria. Mild expression was detected in LCC (C).

In situ hybridization for *MCL-1* mRNA detection

To analyse levels of *MCL-1* mRNA and optimise the target probe, ISH analysis was performed using an MCL-1 probe obtained from Advanced Cell Diagnostics (ACD), designed specifically to target *MCL-1* mRNA. The probe was validated in *MCL-1*-amplified lung adenocarcinoma cells (H1395), and compared with the endogenous control gene *POLR2A* and the negative control *DapB*. Increased levels of mRNA were observed in H1395 cells compared to a non-amplified cell line (H1355). To detect mRNA levels of MCL-1 in tumour samples, the validated MCL-1 probe was applied to 79 NSCLC samples. *MCL-1* mRNA was detected at different levels from mild (0-93) to high (>93) across the 79 analysed samples. High levels were detected in 83% of the amplified cases. Figure 3 shows an example of mRNA levels in an *MCL-1*-amplified tumour, levels of *MCL-1* mRNA in *MCL-1*-amplified tumours were higher than non-amplified.

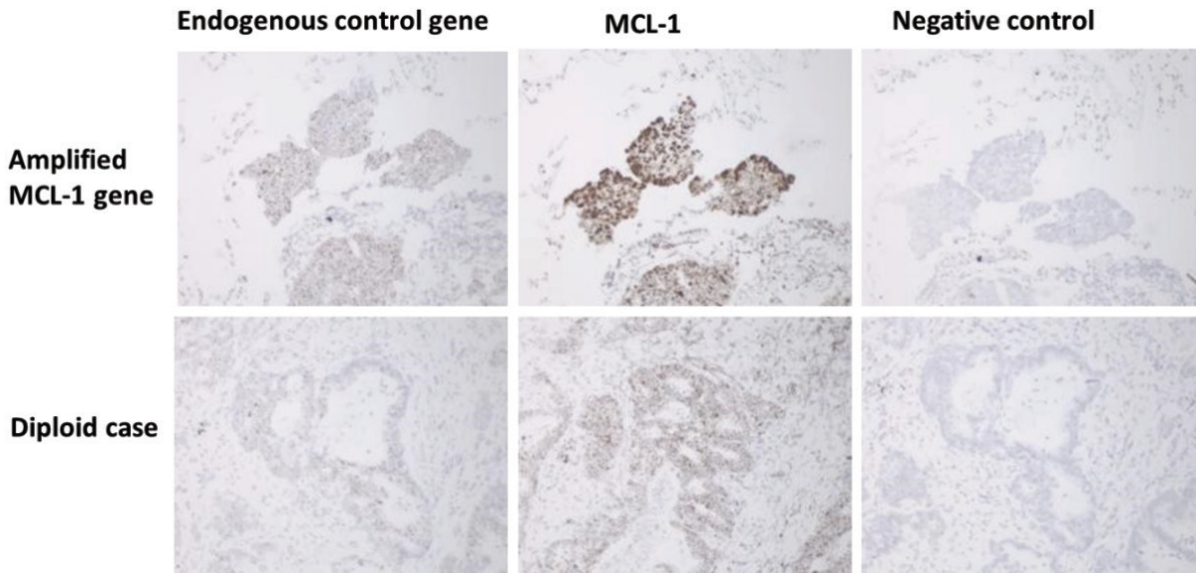

Figure 3. MCL-1 expression in tumour samples. The photomicrograph shows an increased amount of *MCL-1* mRNA in a tumour sample with *MCL-1* amplification, while the diploid case shows normal levels of *MCL-1* mRNA.

Discussion

MCL-1 is a critical gene, and has been implicated in cell survival and drug resistance. Amplification of MCL-1 has been identified in different human malignancies[14] (Leverson *et al*., 2015),with a high frequency in breast and lung cancers [6](Beroukhim *et al*., 2010).In this study, we screened our clinical samples for MCL-1 copy number variation, such as gene amplification and gain, and detected MCL-1 amplification in different histological subtypes of NSCLC. Amplification of 1q26.2 on which MCL-1 is located was detected in ADC in previous studies [6](Beroukhim *et al*., 2010)with a frequency of 20%[15] (Zhang et al., 2015); by contrast, our study has found a frequency of only 2.5% MCL-1 amplification in ADCs. MCL-1 amplification was also identified in SCC [16](Chen *et al*., 2014), and we have also detected *MCL-1* amplification at high frequency in SCC with *MCL-1* gain being mostly associated with ADC. This study is one of the first to indicate that *MCL-1* is commonly amplified in SCC, and gained in ADC (isochromosome), however there is not enough data in the literature to support our findings due to the limitations of similar previous studies. The mitochondrial anti-apoptotic protein MCL-1 is essential for tumour survival in NSCLC[17] (Zhang *et al*., 2011). In previous studies, MCL-1 immunoreactivity was detected in epithelial cells of various tissues such as the epidermis, prostate, endometrium, breast, colon, intestine and respiratory system. Strong staining was detected in the apical layer of differentiated epithelial cells and in the germinal centre of tonsil cells, but not in

the mantle zone[18] (Krajewski *et al*., 1995) . In this study, we also detected strong immunostaining in several normal cell types such as macrophages, the epithelial lining of the respiratory tract and lymphoid cells in the germinal centre of tonsils cells, but not in the mantle or the stratified squamous epithelium of the blind crypt of the tonsil. As MCL-1 is expressed in all differentiated epithelial cells, almost all tumour samples have shown mild to strong staining of (MCL-1)[19] (Whitsett *et al*., 2014). Strong positive and moderate staining of the MCL-1 protein with a clear granular cytoplasmic appearance in the mitochondria was detected. Intense immunoreactivity of MCL-1 was detected in 32.4% of NSCLC with a high proportion in ADC (56%) compared to SCC (44%). Previous studies have also demonstrated that expression of MCL-1 (moderate to strong) is high in ADC (80%) compared with SCC (58%) [19](Whitsett *et al*., 2014). High expression levels of MCL-1 were observed in metastatic melanoma but were not correlated to primary lesions[20] (Zhuang *et al*., 2007), and have also been observed in advanced clinical stages of ovarian cancer[21] (Shigemasa *et al*., 2002); however, there was no correlation between overexpressed MCL-1 and tumour stage in NSCLC.

The MCL-1 protein is known to have a short half-life [4](Wei *et al*., 2012). As a result, mRNA analysis was added to this study. As well as *MCL-1* amplification being analysed using duplex qPCR, the RNA scope technique was used to measure the RNA levels. The advantage of this latter technique is that the RNA molecule is targeted by a specifically designed probe; previous studies have also shown the specificity of RNA scope target probes to different papilloma virus genotypes within different infected cell lines[22] (Wang *et al*., 2015). Moreover, simple conventional chromogenic staining, Haematoxylin dye and bright field microscopy can be used for signal detection. Prior to analysis of *MCL-1* mRNA in NSCLC samples, the ISH technique was applied to an *MCL-1*-amplified cell line (H1395), which showed high sensitivity and specificity. This finding has also been described by Wang and co-workers[23] (Wang *et al*., 2012). High levels of *MCL-1* mRNA (which is a good indicator of gene amplification) was detected in the H1395 cell line and, based on this finding, paraffin-embedded tissues of NSCLC were analysed for *MCL-1* gene expression, which also showed a clear granular appearance of mRNA in the specimens with different expression levels. MCL-1 expression was positively correlated with gene amplification in breast cancer; however, *MCL-1* amplification is not the only factor that controls MCL-1 regulation as several breast cancer tissues shown to be negative for *MCL-1* amplification have demonstrated MCL-1 overexpression[13] (Balko *et al*., 2014). In this study, no correlation was found between MCL-1 protein levels and *MCL-1* amplification. However, a strong correlation was found between *MCL-1* mRNA and gene *MCL-1* amplification, the possible interpretation for which is that the mRNA half-life is longer than the protein half-life[4] (Wei *et al*., 2012).

Conclusions

This study has found that MCL-1 focal amplification was mainly linked to SCC subtype; however, MCL-1 gain was linked to ADC. MCL-1 mRNA is overexpressed in tumour sample with MCL-1 amplification. Although overexpression of MCL-1 protein was very clear in MCL-1 amplified cell line, MCL-1 protein expression was varied among the all analysed tumour samples whether amplified or diploid and this variation could be due to instability of MCL-1 protein or due to tissue processing conditions.

Acknowledgment: I would like to thank Libyan government for supporting and funding this research.

Ethical Clearance: Ethical approval obtained from local Ethic Committee of university of Leicester (United Kingdom).

Source of Funding: Libyan government

Conflict Interest: Nil

References

1- Imielinski M, Berger AH, Hammerman PS, Hernandez B, Pugh T J, Hodis E, et al. Mapping the hallmarks of lung adenocarcinoma with massively parallel sequencing. Cell, 2012 Sep 14;150(6):1107-20.

2- Huncharek M, Muscat J, Geschwind JF. K-ras oncogene mutation as a prognostic marker in non-small cell lung cancer: a combined analysis of 881 cases. Carcinogenesis, 1999 Aug 20, (8):1507-10.

3- Dearden S, Stevens J, Wu YL, Blowers D. Mutation

incidence and coincidence in non small-cell lung cancer: meta-analyses by ethnicity and histology (mutMap). Ann Oncol. 2013 Sep;24(9):2371-6.

4- Wei G, Margolin A A, Haery L, Brown E, Cucolo L, Julian B, et al. Chemical genomics identifies small-molecule MCL1 repressors and BCL-xL as a predictor of MCL1 dependency. Cancer cell. 2012 Apr 17;21(4):547-62.

5- Perciavalle RM, Stewart DP, Koss B, Lynch J, Milasta S, Bathina M, et al. Anti-apoptotic MCL-1 localizes to the mitochondrial matrix and couples mitochondrial fusion to respiration. Nat Cell Biol. 2012 Apr 29;14(6):575-83.

6- Beroukhim R, Mermel CH, Porter D, Wei G, Raychaudhuri S, Donovan J, et al. 2010. The landscape of somatic copy-number alteration across human cancers. Nature. 2010 Feb 18;463(7283):899-905.

7- Vogler, M. Targeting BCL2- proteins for the treatment of solid tumours. Advances In Medicine. 2014 August 27, 1155 (943648):14.

8- Staaf J, Isaksson S, Karlsson A, Jonsson M, Johansson L, Jonsson P, et al. Landscape of somatic allelic imbalances and copy number alterations in human lung carcinoma. International journal of cancer. 2013 May 1, (132): 2020-2031.

9- Nicklas J A, Buel E. Development of an Alu-based, real-time PCR method for quantitation of human DNA in forensic samples. Journal of forensic Sci. 2003 Sep;48(5):936-44.

10- Xu W, Zhai Z, Huang K, Zhang N, Yuan Y., Shang Y, et al. A novel universal primer-multiplex-PCR method with sequencing gel electrophoresis analysis. PLoS One. 2012 Jan 1, 7(1): 22900.

11- Song L, Coppola D, Livingston S, Cress WD, Haura EB. Mcl-1 regulates survival and sensitivity to diverse apoptotic stimuli in human non-small cell lung cancer cells. Cancer Biol & Ther. 2005 Mar 4 (3):267-76.

12- Wesarg E, Hoffarth S, Wiewrodt R, Kroll M, Biesterfeld S, Huber C, et al. Targeting BCL-2 family proteins to overcome drug resistance in non-small cell lung cancer. Int J Cancer. 2007 Dec 1;121(11):2387-94.

13- Balko JM, Giltnane JM, Wang K, Schwarz LJ, Young CD, Cook RS, et al. Molecular profiling of the residual disease of triple-negative breast cancers after neoadjuvant chemotherapy identifies actionable therapeutic targets. Cancer Discov. 2014 Feb;4(2):232-45

14- Leverson J, Zhang H, Chen J, Tahir S, Phillips D, Xue J, et al. Potent and selective small-molecule MCL-1 inhibitors demonstrate on-target cancer cell killing activity as single agents and in combination with ABT-263 (navitoclax). Cell Death Dis. 2015 Jan 15;6(1):e1590.

15- Zhang H, Xue J, Hessler P, Tahir SK, Chen J, Jin S, et al. Genomic analysis and selective small molecule inhibition identifies BCL-X L as a critical survival factor in a subset of colorectal cancer. Mol Cancer. 2015 Jul 2;14:126.

16- Chen Y, Mcgee J, Chen X, Doman TN, Gong X, Zhang Y, et al. Identification of druggable cancer driver genes amplified across TCGA datasets. PloS One. 2014 May 29;9(5):e98293.

17- Zhang H, Guttikonda S, Roberts L, Uziel T, Semizarov D, Elmore S, et al.. Mcl-1 is critical for survival in a subgroup of non-small-cell lung cancer cell lines. Oncogene. 2011 Apr 21;30(16):1963-8.

18- Krajewski S, Bodrug S, Krajewska M, Shabaik A, Gascoyne R, Berean K, et al. Immunohistochemical analysis of Mcl-1 protein in human tissues. Differential regulation of Mcl-1 and Bcl-2 protein production suggests a unique role for Mcl-1 in control of programmed cell death in vivo. Am J Pathol. 1995 Jun;146(6):1309-19.

19- Whitsett TG, Mathews IT, Cardone MH, Lena RJ, Pierceall WE, Bittner M, et al. Mcl-1 mediates TWEAK/Fn14-induced non–small cell lung cancer survival and therapeutic response. Molecular Cancer Research. 2014 Jan 27, 12(4):550-559.

20- Zhuang L, Lee CS, Scolyer R A, Mccarthy SW, Zhang XD, Thompson JF, et al. Mcl-1, Bcl-XL and Stat 3 expression are associated with progression of melanoma whereas Bcl-2, AP-2 and MITF levels decrease during progression of melanoma. Mod pathol. 2007 Apr; 20(4):416-26.

21- Shigemasa K, Katoh O, Shiroyama Y, Mihara S, Mukai K, Nagai, et al. Increased MCL–1 Expression Is Associated with Poor Prognosis in Ovarian Carcinomas. Jpn. J. Cancer Res. 2002 May 93, 542–550.

22- Wang, CY, Deng JY, Cai XW, Fu XL, Li Y, Zhou XY, et al. High EGFR and low p-Akt expression is associated with better outcome after nimotuzumab-containing treatment in esophageal cancer patients:

preliminary clinical result and testable hypothesis. Oncotarget. 2015 Jul 30; 6 (21): 18674–18682.

23- Wang F, Flanagan J, Su N, Wang LC, Bui S, Nielson A, et al. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. The Journal of Molecular Diagnostics. 2012 Jan, 14 (1): 22-29.